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The role of T-regulatory cells and Toll-like receptors in the pathogenesis of human inflammatory bowel disease

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Summary

Two related chronic inflammatory diseases, Crohn's disease and ulcerative colitis, are together often referred to as inflammatory bowel disease (IBD). Current treatment options are not curative, and patients face life-long therapy and debilitation. IBD is thought to be the product of a combination of genetic and environmental factors that result in the abnormal regulation of immune responses. Experimental models have demonstrated that normal $CD4^+$ T-regulatory (Treg) cell responses and commensal bacteria are required for the maintenance of gut immune homeostasis. Recent evidence that $CD4^+$ T cells express Toll-like receptors (TLRs) and respond directly to TLR ligands, suggests that signals from commensal bacteria may directly affect T-cell responses in the gut. In this review, we focus on evidence that defects in Treg cells may underlie IBD in humans. In addition, we discuss evidence that direct signaling via TLRs to T cells can affect IBD and that T-cell-dependent responses to bacterial proteins, such as flagellin, are central to the aetiology of this disease.

Keywords: flagellin; human; inflammatory bowel disease; Toll-like receptor; T regulatory cell

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), known collectively as inflammatory bowel disease (IBD), are autoimmune-like disorders characterized by chronic, idiopathic inflammation of the intestinal mucosal tissue, which causes a range of symptoms including abdominal pain, severe diarrhoea, rectal bleeding and wasting.[1,2] IBD tends to emerge in late childhood, occurs primarily in immunocompetent individuals and is most prevalent in westernized regions of the world.[2,3] CD and UC are distinguished by the tissues affected: CD can affect any region of the gastrointestinal tract in a discontinuous and transmural manner, whereas pathology in UC is restricted to the surface mucosa of the colon, in particular the rectum.[1,2] Current treatment regimens, including anti-inflammatory and immunosuppressive agents, are not curative and only reduce the degree of intestinal inflammation associated with disease.[1]

Although the aetiology of IBD is unclear, both CD and UC are believed to be T-cell-driven processes, with inflammation resulting from inappropriate cytokine production by subsets of $CD4^+$ T-helper (Th) cells.[1] Specifically, CD is associated with Th1 and Th17 cytokine profiles, whereas UC is typically associated with Th2 cells.[1,2] It has been suggested that IBD results from defects in the T-cell-mediated regulatory processes that would normally prevent and/or terminate inflammatory responses; compelling data from mouse models support this hypothesis (reviewed in ref.[4]). In this review, we will focus on evidence that such defects in $CD4^+$ $CD25^+$ forkhead box P3 $(FOXP3)^+$ T-regulatory (Treg) cell function may underlie IBD in humans, and discuss evidence that altered T-cell-dependent responses to bacterial proteins may be central to its aetiology.

Role of Treg cells in human IBD

The gut is an immunologically unique organ that must retain the ability to mount an adaptive response to pathogens while maintaining tolerance to dietary antigens and commensal bacteria.[2] Much experimental evidence

Abbreviations: CBir, C3H/HeJBir; CD, Crohn's disease; CpG, cytosine-phosphate-guanine; FOXP3, forkhead box P3; IBD, inflammatory bowel disease; IFN-γ, interferon-γ; IL, interleukin; LPS, lipopolysaccharide; TCR, T-cell receptor; TGF-β, transforming growth factor-β; Th, T helper; TLR, Toll-like receptor; Treg, T regulatory; UC, ulcerative colitis.

indicates that Treg cells expressing FOXP3 and/or interleukin (IL)-10 have a fundamental role in maintaining gut immune homeostasis.[5,6] Pioneering experiments by Powrie *et al.* demonstrated, more than 15 years ago, that transfer of naive $CD4^+$ T cells into immunodeficient mice in the absence of Treg cells results in colitis that is dependent on the presence of commensal bacteria.[7–9] Remarkably, adoptive transfer of Treg cells not only prevents disease, but also, acting through both IL-10 and transforming growth factor–β (TGF-β)-dependent and -independent mechanisms, halts the progression of established disease and reverses pathology.[5,10] In addition, Treg cells suppress not only T-cell-dependent colitis, but also intestinal inflammation triggered by bacteria.[11] Together, these data provide a reason to believe that methods used to boost and/or restore Treg-cell numbers and function may be effective in the treatment of established IBD in humans.

Many groups have undertaken association studies in patients with IBD to define whether changes in Treg cells can be correlated with disease, with a particular focus on defining differences between circulating cells and gut tissue cells. The vast majority of these studies have analyzed $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells as they can be tracked and are isolated relatively easily. Early studies found that patients with CD or UC have decreased numbers of peripheral suppressive $CD4^+$ $CD45RO^+$ $CD25^+$ $FOXP3^+$ Treg cells during active disease, suggesting that the severity of disease is inversely correlated with the frequency of peripheral Treg cells.[12–15] In contrast, several studies have found that the lamina propria, mesenteric lymph nodes and intestinal inflamed mucosa of patients with either CD or UC actually contain increased numbers of Treg cells compared with healthy controls.[5,13,15,16] These data are consistent with findings in other gut-inflammatory conditions, such as diverticulitis, pseudomembranous colitis and cytomegalovirus-induced colitis, which show a similar correlation between an increase in $FOXP3^+$ Treg-cell numbers and disease severity.[13] Combined with similar data from other autoimmune diseases,[17,18] these findings support the hypothesis that Treg cells traffic to sites of inflammation in an attempt to restore immune homeostasis. Consistent with this notion, a recent study found that the frequency of $FOXP3^+$ cells in the colorectal mucosa decreased following treatment with granulocyte and monocyte adsorptive apheresis and correlated with clinical response.[19]

Notably, *in vitro* functional analysis of Treg cells from the peripheral blood or intestinal mucosal tissue of IBD patients revealed that, despite numerical changes, they maintain normal cell-contact-dependent, cytokine-independent suppressive capacity, even against pathogenic T-effector cells derived from the inflamed mucosa.[20] Furthermore, mucosa-derived Treg cells from IBD patients effectively suppress both proliferation and cytokine production, in contrast to Treg cells from rheumatoid arthritis patients that are reported to specifically lack the capacity to suppress cytokine production.[16,17] These findings from IBD also appear to contrast with a report describing a clear defect in suppressive capacity of cells from patients with multiple sclerosis, although this deficiency was dependent on the strength of T-cell-receptor (TCR) activation,[21] which is known to have a major effect on *in vitro* suppression assays.[22] As studies of Treg cells from human IBD mucosal biopsies have not examined suppressive capacity with varying strengths of T-cell stimulation, it remains possible that subtle functional defects, possibly only in antigen-specific responses, in these cells have been overlooked.

Problems with assessing Treg-cell numbers and function in human inflammatory diseases

Considering the extensive evidence from mouse models, and data from other human autoimmune diseases, it is somewhat surprising that no defect in the numbers or *in vitro* function of tissue-derived Treg cells from IBD patients has been reported. It is important to note, however, that the *in vitro* assays of suppressive function of such cells are performed outside their inflamed environment of origin, and, in the absence of the *in vivo* cytokine milieu, may not accurately reflect their *in vivo* activity. An additional consideration is that in humans, in contrast to mice, FOXP3 is expressed by non-suppressive activated T-effector cells following stimulation, so simple enumeration of $FOXP3^+$ cells is unlikely to assess Treg cell number or function accurately.[23–27] On the other hand, recent data suggest that activated $CD4^+$ T-effector cells which accumulate within inflamed joints do not frequently undergo the demethylation required to express activation-induced FOXP3.[28] If this holds true in the inflamed gut, then activation-induced expression of FOXP3 may not be a major confounding factor in the enumeration of Treg cells in the gut. Finally, recent evidence from murine studies shows that $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells can remain $FOXP3^+$ yet display a Th17 cell phenotype when cultured in the presence of IL-6.[29,30] If human Treg cells have a similar capacity to remain $FOXP3^+$ while acquiring the properties of inflammatory Th17 cells in a pro-inflammatory environment, this would also have a significant impact on the interpretation of many studies that enumerate $FOXP3^+$ cells. Clearly, *ex vivo* analyses of human Treg cells must be accompanied by rigorous functional assays and an extensive phenotypic analysis, including expression of CD127 and intracellular cytokine staining for IL-2 and interferon-γ (IFN-γ), which are not suppressed by activation-induced FOXP3.[23]

Toll-like receptor engagement affects $CD4^+$ T-effector and Treg cells

The importance of Toll-like receptor (TLR)–ligand interactions in dendritic cell biology, and the resulting effects

on adaptive immunity, are well established.[31] More recently, however, it has been recognized that TLRs are expressed on $CD4^+$ T cells, and that direct engagement of TLRs on T cells also affects adaptive immunity (reviewed in refs[32–34]). In the context of IBD, the role of TLRs on $CD4^+$ T cells is of particular interest because commensal bacteria in the gut represent a large source of potential TLR ligands, and loss of integrity of the gut epithelium in IBD patients[35] could result in abnormal exposure of T cells to bacteria-derived innate immune signals, and thereby promote disease.

There are now several reports characterizing the expression profile and function of TLRs on T-cell subsets, and these are summarized in Table 1. In most cases TLR engagement results in enhanced survival and accelerated induction of T-effector-cell responses, with the data relating to TLR-2, -3, -5 and -9 being the most consistent and conclusive. For example, stimulation of TLR-2 with bacterial lipoproteins/lipopeptides induces cytokine production and the proliferation of both murine[36–38] and human $CD4^+$ T cells.[23,37,39] Stimulation of TLR-3, the receptor for viral and synthetic double-stranded RNA, enhances the survival, but not the proliferation, of activated murine $CD4^+$ T cells.[40] Flagellin, the only known ligand for TLR-5,[41,42] enhances TCR-stimulated proliferation and cytokine production from human T-effector cells.[39,43,44] Similarly, activation of TLR-7/-8, the receptor for viral single-stranded RNAs and nucleosides, stimulates memory $CD4^+$ T cells to increase IFN-γ production.[39] Finally, activation of TLR-9, the ligand for unmethylated cytosine-phosphate-guanine (CpG) motifs from bacteria, directly co-stimulates murine $CD4^+$ T cells.[45]

Stimulation of TLRs can also directly influence the function of Treg cells.[46] For example, using mice deficient for TLR-2 or MyD88, an intracellular adaptor protein known to be essential for many TLR-mediated effects, it was shown that stimulation of TLR-2 on Treg cells, in the context of IL-2 and TCR triggering, induces proliferation and a transient loss of FOXP3 expression and suppressive capacity.[47,48] In apparent direct contrast, exposure of human Treg cells to endogenous heat-shock protein 60, which also stimulates TLR-2, enhances their suppressive activity.[49] TLR-5 is also expressed by human Treg cells at levels similar to those on $CD4^+$ T-effector cells, and stimulation with its ligand, flagellin, enhances FOXP3 expression and suppressive capacity.[43] In contrast to the effects of TLR-5, triggering TLR-8 on human Treg cells reverses suppression, while $CD4^+$ T-effector cells remain unaffected.[50] Similarly, stimulating TLR-9 acts directly on Treg cells to block suppression.[51] In contrast, Tregs from *tlr-9−/−* mice are impaired in their ability to suppress an *in vitro* assay of autoreactivity in a lupus model, suggesting that TLR-9 may provide a positive signal to Treg cells in this context.[52] An as-yet unanswered question is whether Treg cells differ from T-effector cells in terms of whether they are receptive to TLR signals independently from TCR engagement, or at very low concentrations of antigens.

There is significant controversy regarding the role of direct signalling via TLR-4 to T cells. In some cases lipopolysaccharide (LPS) (recognized by TLR-4) was reported to stimulate the activation of protein kinase C in $CD3^+$ T cells[53] and perforin production in a subset of $CD4^+$ T cells in patients with ankylosing spondylitis.[54] Moreover, Fukata *et al.*[36] found that LPS-stimulated proliferation of T-effector cells was dependent on Myd88. In contrast, others found no effect of LPS on $CD4^+$ T-effector cells.[37,43,47] Whether or not LPS/TLR-4 interactions influence Treg cells is also controversial. Initial reports showed that LPS directly enhances suppression by murine Treg cells through TLR-4;[55] however, subsequent studies found the expression level of TLR-4 on murine Treg cells to be low.[40,47,48] With respect to human Treg cells, two studies found that these cells neither express TLR-4 nor respond to LPS,[37,43] whereas another report suggests that Treg cells do express TLR-4 and that LPS stimulation results in decreased FOXP3 expression.[56] The mostly likely explanation for these varying results is differences in the purity of the LPS used (commercial sources are often contaminated with TLR-2 ligands) as well as the purity of the cells assayed, which must be rigorously purified to ensure no contaminating antigen-presenting cells could be activated in response to LPS and thereby provide co-stimulation and cytokines.

Role for altered TLRs in IBD

TLRs are expressed on a large variety of immune cells and play a major role in initiating immune responses. In the steady state the gut is a rich source of TLR ligands from commensal bacteria, and during disease a variety of endogenous TLR ligands may also be released in the process of inflammation and tissue destruction. TLRs thus have dual roles in IBD: they are necessary for maintaining tolerance and eliminating pathogenic microorganisms, but they can also amplify inappropriate immune responses that ultimately cause chronic inflammation. The vast majority of studies have considered the role of TLRs in IBD in terms of their effects on innate immune cells, such as dendritic cells and macrophages (reviewed in ref.[57]). Recently, however, it has been demonstrated that specific ligation of TLRs on T cells can also influence the development of colitis. Pathogenic T-effector cells from Myd88-deficient mice have a significant defect in Th17 cell differentiation *in vivo* and do not induce colitis when transferred into immunodeficient mice.[36,58] Moreover, *myd88−/−* Treg cells are less able to suppress the adoptive transfer of colitis,[36] and treatment of mice with a TLR-9 ligand induces the development $CD4^+$ Treg cells that are protective in the T-cell transfer model of colitis.[59] These

Table 1. Summary of the expression pattern and function of Toll-like receptor (TLR)1–10 on mouse and human $CD4^+$ T-cell subsets

	Human				Mouse			
	T-effector cells		T-regulatory cells		T-effector cells		T-regulatory cells	
	Expression	Biology	Expression	Biology	Expression	Biology	Expression	Biology
TLR-1	Expressed, mRNA[39,90,91]	ND	ND	ND	Expressed, mRNA[55,58]	ND	Expressed mRNA[55,92]	ND
TLR-2	Expressed, mRNA;[39,90,91] expressed in memory cells;[37] not expressed mRNA and protein;[93]	Increased proliferation and cytokine production in memory cells[23,37,39]	Not expressed, mRNA and protein[93]	Hsp60 enhanced suppression[49]	Expressed, mRNA and protein[36,48,55]	Increased proliferation any cytokine production,[36,37,48] specific effect on Th1, not Th2 cells[38]	Expressed, mRNA[55,94]	PAM3Cys increased proliferation, transient decrease in FOXP3 expression and suppression,[47,48]
TLR-3	Expressed mRNA[39,90]	No biological effect detected[39]	ND	ND	Expressed, mRNA and protein[36,40,55]	Enhanced survival and proliferation[36,40]	Expressed, mRNA[55]	ND
TLR-4	Expressed mRNA,[39,90] not expressed mRNA and protein[43,93]	No biological effect detected[37,39,43]	Expressed mRNA[43], not expressed protein[43,93]	No biological effect detected[43]	Expressed, mRNA and protein[36,55]	Enhanced proliferation[36]	Expressed, mRNA[55]	Increased proliferation,[55] no biological effect detected[37,47]
TLR-5	Expressed; mRNA and protein[39,43,90,91,93]	Enhanced proliferation and cytokine production[39,43,44]	Expressed, mRNA and protein[43,93]	Enhanced FOXP3 expression and suppressive capacity[43]	Expressed, mRNA;[40,55] not expressed, mRNA[58] or protein[36]	ND	Expressed, mRNA[55]	ND
TLR-6	Expressed mRNA,[90] not expressed mRNA and protein[39,93]	ND	Expressed, mRNA and protein[93]	ND	Expressed, mRNA[55,58]	ND	Expressed, mRNA[55]	ND
TLR-7	Expressed mRNA[39,90,91]	Enhanced proliferation[39]	Not Expressed mRNA[50]	ND	Expressed, mRNA[55,58]	ND	Expressed. mRNA[55]	ND
TLR-8	Expressed mRNA,[90] not expressed, protein[39,93]	No biological effect detected[50]	Expressed mRNA protein[50,93]	Reduced suppressive capacity[50]	Expressed, mRNA[55,58]	ND	Expressed, mRNA[55]	ND
TLR-9	Expressed, mRNA[39,90,91]	Expression down-regulated upon infection[91]	Not Expressed, mRNA[50]	ND	Expressed, mRNA and protein[36,40,95]	Increased proliferation[36,40,51] and Bcl-x(L) expression[95]	Not expressed, mRNA[55]	Blocks suppressive function;[51] necessary for suppression in a lupus model[52]
TLR-10	Expressed, mRNA,[90,91] not expressed, mRNA and protein[39,93]	ND	Expressed mRNA and protein[93]	Expression regulated by FOXP3[93]	ND	ND	ND	ND

T-regulatory refers to cells defined on the basis of CD25 and/or forkhead box P3 (FOXP3) expression. T effector refers to cells that were either total $CD4^+$ T cells, or $CD4^+$ T cells that were depleted of T-regulatory cells. ND, not determined as of July 2008.

results suggest that T-cell-specific stimulation of TLRs, presumably by commensal bacteria, has a significant role in the development of IBD.

Additional evidence for the role of TLRs in IBD comes from studies of flagellin and TLR-5. TLR-5-deficient mice, originally described by Uematsu *et al.*,[60] develop spontaneous colitis,[61] suggesting that flagellin–TLR-5 interactions normally have a protective anti-inflammatory role. In contrast, in humans, a common polymorphism in TLR-5 that produces a dominant-negative receptor is protective against CD, but not UC, in people of Ashkenazi Jewish descent.[62,63] The reason for this apparent contradiction is unclear, but could relate to differences between mice and humans in species of commensal bacteria, roles of the intracellular sensor of flagellin [the nucleotide-binding oligomerization domain (NOD)-like receptor known as Ipaf],[64] effects of TLR-5 on $CD4^+$ T cells and/or differences in the expression of TLR-5. Differences between mouse and humans in expression of TLR-5 may be of particular importance, as studies have shown that in mouse intestinal tissue only $CD11c^{hi}$ $CD11b^{hi}$ lamina propria dendritic cells express TLR-5,[65] whereas in humans, TLR-5 is expressed on a variety of cells including intestinal epithelial cells,[42] dendritic cells,[66] endothelial cells,[67] $CD4^+$ T-effector cells and Treg cells.[43]

In addition to TLR-5, polymorphisms in the TLR-1, -2, -4, -6 and -9 loci have been associated with IBD in humans,[68–70] although the functional effects of these variants are not well defined. In terms of protein expression, TLR-3 is down-regulated, whereas TLR-2 and -4 are up-regulated, in the intestinal mucosa of patients suffering from active IBD.[71,72] *In vitro* evidence suggests that altered TLR-2 signalling is associated with disease because peripheral blood mononuclear cells isolated from patients with active CD or UC produce more TNF-α in response to TLR-2 stimulation than those of healthy controls.[73] There is also genetic evidence that functional polymorphisms in the intracellular sensor for bacterial peptidoglycan, NOD2 (also known as CARD15), is linked to CD,[2] but there is currently no evidence regarding a possible role for these receptors in T cells.

Role for bacterial antigens in IBD

There is strong evidence that abnormal regulation of the immune response to commensal bacteria is a driving force in IBD (reviewed in ref.[74]) Mouse models of IBD have shown that the development of colitis is dependent on the presence of gut flora because the disease is generally absent in mice raised in germ-free environments and is ameliorated by the administration of antibiotics[75] in normal laboratory conditions. Moreover, in IL-10-deficient mice, the development of spontaneous colitis requires commensal flora and an intact TLR–MyD88 signaling pathway.[76] More direct evidence for the antigenic role of commensal flora comes from studies of immune responses towards specific gut bacterial antigens. For example, spontaneously colitic C3H/HeJBir mice (CBir), generated by selective breeding of C3H/HeJ mice for the colitic phenotype, show increased B-cell and T-cell reactivity towards bacterial antigens,[77] and transfer of $CD4^+$ T cells from colitic CBir mice causes colitis in immunodeficient recipient mice.[78,79] This response is specific for commensal flora because the relevant T cells do not respond to food antigens or faecal extracts from germ-free mice.[80]

A variety of clinical observations in IBD patients suggest that responses to commensal bacteria are also central to the development of human disease. Most notably, IBD lesions are preferentially found in the terminal ileum and colon, which harbour the highest concentrations of bacteria. In addition, altering commensal flora with antibiotics or probiotics can reduce the symptoms associated with disease, albeit transiently.[81] Similar to colitic mice, human IBD patients show enhanced immune responsiveness to gut bacterial antigens and concurrent loss of tolerance.[82,83]

Some of the specific bacterial antigens driving disease in mouse models of colitis and human IBD have recently been identified. One key antigen that drives disease is flagellin, the major structural subunit of bacterial flagella (reviewed in ref.[42]). Initially, flagellin from commensal *Clostridia* species was found to be an antigen in the CBir mouse model of spontaneous colitis. Remarkably, when sera from CBir mice were used to probe a caecal bacterial phage display library, 25% of all proteins cloned were flagellins from commensal organisms.[84] More detailed analysis revealed that CBir mice have antibody responses to the amino-terminal conserved domain of flagellin, and that flagellin-specific $CD4^+$ T-effector cells can induce severe colitis.[84] Remarkably, even in wild-type mice, chemically induced colitis is associated with the development of anti-flagellin immunoglobulin responses, indicating that flagellin can be a dominant antigen in both spontaneous and experimentally induced colitis.[85,86] Immune responses to flagellin are also implicated in human IBD:[84,87–89] subsets of patients with CD, but not with UC, have elevated antibody responses to CBir flagellin,[84,88] as well as to a wider variety of flagellin subtypes.[87,88] That flagellin is the only known human TLR ligand which is a protein, and can thus activate both the innate and adaptive immune systems in parallel, may be one reason why this protein is a dominant antigen in IBD.

Concluding remarks and a model

It is clear that multiple defects in innate and adaptive immunity can initiate and sustain IBD. Here we have focused on evidence as to whether insufficient numbers or function of Treg cells could be a primary cause of

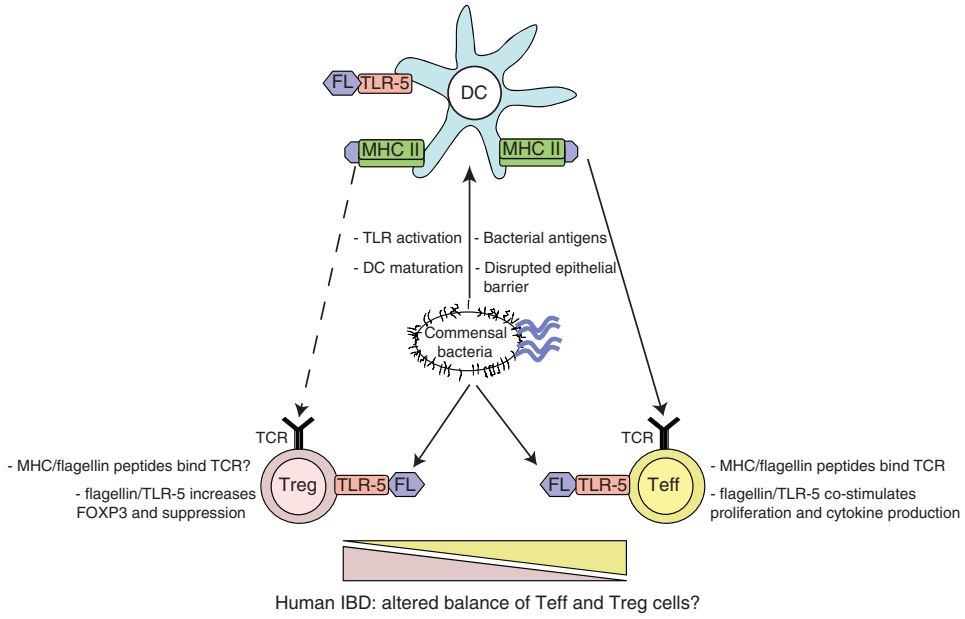

Figure 1. Model for how flagellin could influence the balance between T–effector (Teff) and T-regulatory (Treg) cells in inflammatory bowel disease (IBD). Increased epithelial permeability associated with IBD could result in abnormal exposure of cells in the lamina propria to flagellin from commensal bacteria. Flagellin could be phagocytosed and presented as an antigen by dendritic cells (DCs) to $CD4^+$ T cells. In parallel, flagellin could stimulate Toll-like receptor 5 (TLR-5) on immune and non-immune cells to induce maturation, proliferation and cytokine production. Depending on the concentration, and on other inflammatory signals, flagellin could thereby alter the balance between Treg and Teff cells. Other TLR ligands from commensal bacteria may also directly stimulate TLRs on T cells and contribute to the overall effect. FL, flagellin; FOXP3, forkhead box P3; MHC, major histocompatibility complex; TCR, T-cell receptor.

IBD. Despite overwhelming data from animal models that alterations in the numbers or function of Treg cells cause colitis,[4] it has been difficult to detect significant defects in peripheral or gut Treg cells in association with UC or CD in humans. These *in vitro* studies of immune regulation with human cells, however, have been limited by inadequate markers to track and isolate Treg cells, and an inability to replicate the *in vivo* inflammatory environment. There is therefore an urgent need to develop better assays with which to study the *ex vivo* Treg cells isolated from inflammatory environments and to identify markers that are specifically associated with function. If there is a real deficiency in Treg-cell function in human IBD, then cell therapy-based strategies similar to those that are effective in mice[5,10] could be developed.

Of particular interest in the context of IBD is how TLR ligands and antigens derived from commensal bacteria may directly influence the balance and function of T-effector and Treg cells. Via its capacity to act as an antigen,[84] an adjuvant both for T-cell and antibody responses[85] and as a co-stimulatory molecule,[43] the TLR-5 ligand flagellin appears to be central to this process (Fig. 1). We can speculate that the increased epithelial permeability associated with IBD could result in abnormal exposure of T cells in the lamina propria to flagellin from commensal bacteria. Flagellin could be phagocytosed and presented as an antigen by dendritic cells and, in parallel, stimulate TLR-5 on immune and non-immune cells. Evidence that mutations in TLR-5 may protect humans from CD[62,63] suggests that pharmacological inhibition of this pathway may be therapeutically beneficial. Notably, because expression of TLR-5 in humans is much more widespread than in mice,[85] parallel studies in mice and humans will be needed to define the clinical relevance of data from models of IBD.

With respect to flagellin acting as a TLR ligand on $CD4^+$ T cells, low concentrations enhance the expression of FOXP3 and the suppressive capacity of Treg cells, whereas high concentrations stimulate T-effector cell function.[43] Presumably, in active IBD, the latter situation would prevail and perpetuate the loss of normal Treg-cell function. Whether or not flagellin also has a role as an antigen in altering the balance of Treg and T-effector cells in this context remains to be defined. However, we speculate that the induction of antigen-specific tolerance to flagellin by promoting the development of flagellin-specific Treg cells may be an attractive approach to restoring intestinal regulatory responses and contribute to the treatment of IBD.

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